

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Simon R.Green and Roger Neil Sleigh

Application No. 10/581,585

Confirmation No. 7005

Filed: April 20, 2007

Art Unit: 1623

For: COMBINATION OF CDK INHIBITOR AND
CS-682 OR A METABOLITE THEREOF

Examiner: Eric Olson

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR §1.132

I, Simon R. Green, Ph.D., declare:

1. I am presently a Program Manager at Cyclacel Pharmaceuticals, Inc. I have extensive research experience in the areas of cancer biology and medicinal chemistry, as demonstrated by my curriculum vitae (attached as Exhibit A).
2. I have carefully read and understand the contents of United States Patent Application No. 10/581,585. It is my understanding that the invention set forth in the pending claims of the above-identified patent application relates to combinations of compounds, and pharmaceutical products, which are useful for the treatment of proliferative disorders, and methods and uses thereof. It is my opinion that the subject matter of the invention is novel and non-obvious in view of the art that was available at the time of the filing of the application, including the art cited in the Office Action dated May 7, 2009.
3. Attached as Exhibit B are details of a flow cytometry assay, including two tables of data representing the percent of apoptotic cells (sub-G1 cells) within the total cell populations

following 72 hr treatment with seliciclib (R-roscovitine) or CNDAC (1-(2-C-Cyano-2-deoxy- β -D-arabino-pentafuranosylcytosine) or the sequential combination of both agents.

4. Also presented in Exhibit B are details of a corroborating H2AX assay, wherein H358 cells were treated for 24 hr with CNDAC (0.5 μ M) followed by 72 hr with seliciclib (3 μ M) (or the equivalent period with media for the single agent controls). Cell lysates were analyzed via Western blots, and the blots were probed with an antibody raised against phospho-histone H2AX as a marker of double-stranded DNA breaks.

5. In order to demonstrate that the combination of CNDAC and seliciclib is synergistic, two experimental studies are presented, summarized in Exhibit B. First, cells were treated with either the single agents, or the combination, and then analysed by flow cytometry for the appearance of apoptotic cells, *i.e.* cells that have a sub-G1 DNA content. Surprisingly, combinations of CNDAC and seliciclib resulted in effects that were synergistic, *i.e.*, greater than the sum of the effects of either compound alone.

Next, cells were treated with either the single agents or the sequential combination for 96 hr (CNDAC prior to seliciclib). Cells were then tested for double-stranded DNA breaks. At doses known to induce synergistic cell death by flow cytometry analysis, it was apparent that the combination generated a clear increase in the amount of double stranded DNA breaks. This data suggests that seliciclib is capable of enhancing CNDAC induced DNA damage.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made may be punishable by fine or imprisonment, or both, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: 30th Oct. 2009

Signature: _____



Exhibit A**Simon Richard Green**
CURRICULUM VITAE**SUMMARY**

An individual with fifteen years experience in the biotechnology industry primarily focussed in the oncology and anti-infective disease areas. Extensive project/program management experience covering all areas of the drug discovery process from target identification to clinical development. Recently engaged in translational research for compounds in clinical development and acting as the primary liaison between the research group and clinical department.

CAREER EXPERINCE**3/02 - present Cyclacel Ltd. UK: Seliciclib Program Manager/Translational Research Leader.**

- Asked by Cyclacel to lead Translational Research Program
- Managed up to nine direct reports and coordinated numerous external collaborations
- Acted as the main liaison between Cyclacel's Research and Clinical Development groups
- Devised and instigated the translational studies on Cyclacel's three clinical programs: seliciclib - a CDK inhibitor; sapacitabine - a novel DNA damaging agent and CYC116 - an aurora inhibitor
- Seliciclib program manager coordinating the ongoing development of this clinical program
- Coordinated the submission of an Investigator Led Study to the Spanish Clinical Regulatory Authorities and the Hospital's IRB
- Coordinated the late stage preclinical development of the seliciclib follow-on program including design and supervision of external *in vivo* studies

9/00 - 3/02 Cyclacel Ltd. Dundee UK: Program Manager Polgen/Plk1.

- Acted as the scientific liaison between the two Cyclacel sites: Cambridge (Target Identification) and Dundee (Drug Discovery)
- Devised strategy for RNAi-based HTS to identify mitotic genes in *Drosophila* cell lines
- Devised selection criteria for novel mitotic targets identified at Cyclacel
- Presented scientific program to companies for collaborative exploitation of the technology
- Established Plk1 as first selected target and ran the initial Discovery project team

2/99 - 9/00 Fluorescence Ltd. Leeds, UK: Group Leader Molecular and Cellular Biology.

- Developed strategy to exploit core technology at start-up company exploiting novel fluorescent assay technology
- Presented scientific component of company presentations to external investors
- Managed a group of three direct reports

1/93 - 2/99 RiboGene, Inc., CA, USA: HIV and Anti-Fungal Project Leader.

- Designed and established research program at the conception of a start-up biotech company
- Developed seven assays, some for high-throughput *in vitro* screening and others were yeast or mammalian cell cell-based

- Managed a group of five direct reports; coordinated a team of thirteen people on the project
- Directed and managed collaborations with two pharma. companies (Abbott and Parke Davis)
- Funded projects by writing NIH grants (~\$1M obtained)

2/90 - 1/93 Cold Spring Harbor Laboratory, NY, USA. Postdoctoral fellow: Structure and function of the interferon induced protein kinase DAI.

- Exposed to an intensive work environment at a world leading research centre
- Two different projects related to the activity of a kinase that regulates protein synthesis
- Identified novel RNA binding motif which has since been found in a number of other proteins

9/86 - 9/89 University of Kent, UK. Ph.D. research project: The molecular analysis of eukaryotic initiation factor 2 α .

- Trained as a molecular biologist, cloning and expressing a higher eukaryotic translation factor in the yeast *Saccharomyces cerevisiae*.

PUBLICATIONS - 24 published manuscripts (available on request)

ABSTRACTS - 42 meeting abstracts (recent ones available on request)

PATENTS - One granted and 15 applications submitted

CORE SKILLS

- Originally trained as a molecular biologist
- Fifteen years experience in the biotech. industry in the areas of oncology and anti-infectives
- Broad knowledge of the cancer therapeutic field particularly related to the core areas of Cyclacel's drug development programs
- Extensive project/program management experience including one clinical program
- Liaison with both academic and pharmaceutical collaborators
- Well versed with Microsoft Word, Excel & PowerPoint packages
- Information dissemination through report writing, manuscript preparation and presentations
- Staff supervision including developing approaches to improve staff retention

PERSONAL INFORMATION

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EDUCATION

1982 - 86 University of Bath
B.Sc. Applied Biology, 2i (Hons)
Specialisation Microbiology
1986 - 90 University of Kent
Ph.D. Biochemistry

REFERENCES - available on request

EXHIBIT BFlow Cytometry Assay

Cells (H460, H358) were seeded in 10 cm plates at approximately 5×10^5 cells/plate and left to settle for 2 hr. Cells were treated 24 hr with one agent, washed and then treated for 72 hr with the second agent. The treatments used were: drug free media (for single agent controls), 1 x IC₅₀ seliciclib, 1 x IC₅₀ CNDAC or both drugs sequentially. After the compound incubations were completed, media was removed, cells were harvested by trypsinisation, washed twice in PBS and then fixed in 70% (v/v) ethanol at -20°C for 16 h. Cells were washed twice in PBS containing 1% (w/v) BSA, then incubated with 50 µg/ml propidium iodide and 50 µg/ml ribonuclease A for 20 min at room temperature. Cells were analysed for DNA content by flow cytometry using CellQuest (BD) on a BD LSR flow cytometer. The tables below represent the percent of apoptotic cells (sub G1 cells) within the total cell population following 72 hr treatment with seliciclib, or CNDAC, or the sequential combination of both agents.

H460 Cells

Treatment	Sub-G1
Control	3.2
Sel/media	3.0
media/CNDAC	9.8
Sel/CNDAC	17.2
CNDAC/media	13.2
media/Sel	8.9
CNDAC/Sel	31.9

H358 Cells

Treatment	Sub-G1
Control	3.2
Sel/media	3.5
media/CNDAC	13.3
Sel/CNDAC	18.2
CNDAC/media	18.7
media/SL	8.7
CNDAC/Sel	28.2

H2AX Assay

As an extension of the *in vitro* studies assessing the synergistic effects of the combination of CNDAC and seliciclib, cells were treated with either the single agents or the sequential combination for 96 hr (CNDAC prior to seliciclib). After incubation, the cells were harvested, lysates prepared and the number of double-stranded DNA breaks evaluated by western blot analysis using an antibody raised against phospho-histone H2AX (an established marker of dsDNA breaks). This was evaluated in three NSCLC cell lines, using a range of doses of both agents. At doses known to induce synergistic cell death by flow cytometry analysis, it was apparent that the combination generated a clear increase in the amount of ds DNA breaks (Figure 1). This effect was not observed at drug concentrations that were not synergistic. This data would suggest, that seliciclib is capable of enhancing CNDAC induced DNA damage.

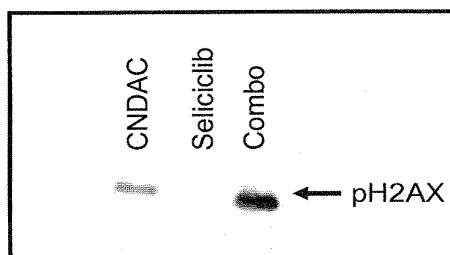


Fig 1. Western blot of H358 cell lysates treated for 24 hr with CNDAC (0.5 μ M) followed by 72 hr with seliciclib (3 μ M) (or the equivalent period with media for the single agent controls). Blots were probed with an antibody raised against phospho-histone H2AX as a marker of double-stranded DNA breaks.